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THE FRACTIONAL LIQUEFACTION OF RICE STARCH

BY

F. J. WARTH, M.Sc
Agricultural Chemist, Burma

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D. B. DARABSETT, B.Sc
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NOTE

In a former paper* it was shown that rice varieties could be roughly distinguished by disintegrating the grains with Alkali.

In the present paper a new method is described by means of which rice varieties can be distinguished from one another by differences in the quality of their starches.

It is shown that the cooking quality of rice is correlated with starch quality.

The paper consists of two parts.

In the first part the method of fractional liquefaction for comparing rice starches as devised and tested by me is described.

The second part contains an application of the method to the differentiation of the starches of seven common rice varieties of Burma. This part of the work has been carried out in co-operation with Mr. Darabsett.

F. J. WARTH,

Agricultural Chemist, Burma,

Mandalay.

* Warth and Darabsett, *Pusa Agri. Res. Inst., Bull.* 38.

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PART I

FRACTIONAL LIQUEFACTION OF RICE STARCHES

Starches of different cereals are generally distinguished from one another by the size, shape and optical properties of the granules.

It has been shown that they may also be distinguished by their unequal resistance towards amylolytic enzymes and in possessing different liquefaction (or so-called gelatinisation) temperatures.

Among the numerous publications showing the differential effect of enzymes upon different starches, the following by Stone[1], J. O'Sullivan[2] and Wolff and Fernbach[3] are perhaps the most important.

Among the earlier determinations of the liquefaction temperatures of starches Lippmann's[4] figures were obtained by

1 Stone, W. E. *U. S. Agri. Dept., Office Expt. Stas. Bull.* 34, 1896.

2 O'Sullivan, J. *J. C. S. Trans.*, 1904, p. 616.

3 Wolff and Fernbach, *Compt. Rend.* 1907, 144, p. 645.

4 Lippmann quoted by König. *Chemie der menschlichen N. & G. Mitt.*

observing with the aid of the microscope the points of incipient and complete rupture of the granules.

This method has lately been utilised for the same purpose by Nyman[5] who used the micropolariscope and for end point observed the disappearance of the interference crosses.

Nyman found that the younger granules were more easily ruptured than the older.

He showed also that by prolonging the action of heat the process of liquefaction took place at a lower temperature.

C. J. Lintner[6] studied the action of diastase on ungelatinised starch at different temperatures.

His work was continued under slightly modified conditions by Von Sigmond[7] who showed that considerable differences existed in the liquefaction temperatures of different starches.

More recently it has been pointed out by Wolff and Fernbach[8] that the starch of the pea consists of two distinct fractions.

In commencing work on the starches of rice varieties it had to be assumed that the differences to be looked for (if indeed any existed) would not be so marked as those found by experimenters who compared starches derived from very different sources.

As the methods employed by previous workers were not likely to be sufficiently precise to enable measurements to be made of relatively small differences some new method had in the first place to be devised.

I decided to compare the starches by a process of fractional liquefaction at different temperatures.

After various trials the following method was adopted.

A definite weight (usually 1 gramme) of the finely divided substance is stirred up with 70 c.c. of water and maintained at the desired temperature for one hour with regular stirring.

5 Nyman, *Z. Unters, N. S. G.* 1912.

6 Lintner, C. J.—*Woch-Br.* 8-22 *and Chem. Centbl.*, 1890-1—509.

7 Von Sigmond. *Chem. Centbl.*, 1897-2—614.

8 Wolff and Fernbach—*Compt. Rend.*, 1905, 140.

All the starch liquefiable at that temperature is thereby converted into a paste in which the unaltered starch granules requiring a higher temperature for liquefaction remain suspended.

The liquid is cooled, water added to compensate for evaporation, 10 c.c. malt extract are run in and the conversion allowed to proceed for two hours with regular stirring at 30° C.

The unaltered starch granules presumably remain intact whilst the paste is dissolved forming maltose and dextrin. That erosion of starch granules does not take place to a measurable extent under the conditions of the experiment will be seen in the sequel.

The liquid is made up to a definite volume and filtered with kaolin.

An aliquot portion of the filtrate is hydrolysed with hydrochloric acid and the glucose content determined by Fehling solution using permanganate for estimating the cuprous oxide. From this figure the amount of starch liquefied at a given temperature may be calculated.

30° C was chosen as a convenient temperature for all conversions with malt because it is sufficiently low to give practically no erosion whilst it is high enough to allow complete conversion and to prevent the possibility of reversion.

The method resembles somewhat that employed by Lintner (loc. cit.), but as the object of the work differs so essentially from Lintner's enquiry one important distinction in the procedure should be remarked upon.

With the object of making the effect of the malt constant throughout, all the malt conversions in the present work were carried out at the same temperature.

Lintner malted at different temperatures because his intention was rather to study the effect of temperature upon malting.

The present enquiry was commenced before the publication of Nyman's paper, but the time factor noted upon by him was fortunately foreseen and allowed for as well as possible by maintaining a constant temperature for liquefaction for a much longer time than is usuall considered necessary.

It has indeed not been proved that a still longer exposure would not yield still higher results, but one hour has given concordant duplicate figures which have a definite value, whatever future work on this time factor may reveal.

The method as described in detail above can be applied to starch samples without any difficulty, but in the present problem dealing with rice grains there is the preliminary difficulty of disintegrating the grains. This must be satisfactorily overcome before the method can be applied at all.

It has been shown by Brown and Heron that by protracted grinding starch granules become completely soluble in malt without previous liquefaction.

Therefore, to cause as little damage as possible by grinding, the grain was, in the first series of experiments, ground up with successive small quantities of water which were poured off when turbid.

The following experiment will show the extent of breakage to be expected.

Two varieties of rice were given—

(*a*) the ordinary grinding with water as just described;

(*b*) a dry grinding for 45 minutes followed by wet grinding.

The samples thus obtained were liquefied at 30° and then converted at 30° with malt. The following results were obtained.

	Wet grinding.	Excessive grinding.
Sample I	6·8 c.c. ($KMnO_4$) Potassium Permanganate.	11·7 c.c. ($KMnO_4$) Potassium Permanganate.
Sample II	3·66 c.c. ($KMnO_4$) Potassium Permanganate.	7·7 c.c. ($KMnO_4$) Potassium Permanganate.

A low liquefaction temperature was chosen to bring out the effect of the grinding. It would, of course, become obscured by liquefaction at a higher temperature.

We see that the excessive grinding caused an increased liquefaction of starch, the figures being roughly twice as high as those obtained by wet grinding.

With the aid of this process of wet grinding for preparing samples, liquefaction determinations were carried out on three rice samples and one starch sample.

The grinding for each determination was done separately and as a check a duplicate determination (also with separate grinding) was made, using 20 c.c. malt.

The results of this work are shown in the accompanying table. The figures under A & B refer respectively to the results obtained with 10 and 20 c.c malt.

Percentage Liquefaction of Starch.

	30°		40°		50°		60°		70		80°	
	A	B	A	B	A	B	A	B	A	B	A	B
No. 1.	21	21	20	19	23	26	60	57	69	71	67	67
No. 2.	13	13	9	10	10	12	15	22	57	62	58	59
No. 3.	12	11	9	11	11	12	19	18	59	59	61	61
Starch	0	0	0	0	3	3	29	25	71	73	76	76

No. 1 is a glutinous rice. Nos. 2 and 3 are fairly hard non-glutinous rices.

The figures show clearly that the method when applied to starch gives concordant and interesting results.

From the starch sample figures for 30°, 40°, and 50° it is evident also that no measurable erosion of unliquefied starch granules occurs under the conditions of the experiment.

We see also that the glutinous rice starch liquefies at a lower temperature than the starches of the non-glutinous rices.

The amounts of liquefaction at lower temperatures are, however, very high when compared with the starch sample which required no grinding.

It is evident, therefore, that grinding, even by the wet process, causes excessive breakage and makes the determinations at lower temperatures valueless.

Some other means of disintegration had therefore to be found.

The use of alkali for this purpose has obvious difficulties, but it was found possible eventually to disintegrate the grains satisfactorily with Potassium Hydrate in the following manner.

The weighed polished seed is placed for 24 hours in 10 c.c. of 1 % Potassium Hydrate. The liquid is then made faintly acid and poured off into a beaker.

The seed is gently ground in a mortar with successive small quantities of water and the starchy liquid added to the beaker of acid liquid. Neutralisation is effected with Calcium Carbonate suspended in water.

The specimen is then ready for liquefaction and conversion as usual.

The above three rices treated in this way yielded the following results :—

	30°	40°	50°	60°
No. 1	7·6	6·5	8·3	65·5
No. 2 ..	3·6	3·4	3·7	5·7
No. 3	3·0	3·2	3·5	19·8

These figures show that by means of the above alkali process the seeds can be satisfactorily disintegrated without causing appreciable breakage of the starch granules.

PART II

A COMPARISON OF THE STARCHES OF 7 RICE VARIETIES

The specimens examined were pure line cultures of well known types.

We disintegrated the polished seeds by the Potassium Hydrate process and carried out the fractional liquefaction determinations exactly as detailed above.

The accompanying table shows the results obtained.

TABLE I. *Fractional Liquefaction of Starch.*

Sample.	Grammes starch liquefied per 100 grm. seed at							Total* Starch.	Proteid.
	50°	55°	60	65	70°	75°	80		
No. 1	3·8	5·2	11·7	45·2	65·0	68·1	69·3	74·5	7·08
No. 2	2·4	4·7	2·9	8·8	24·2	65·2	68·1	73·4	7·18
No. 3	5·9	8·6	23·7	59·3	62·8	64·4	66·7	73·4	7·69
No. 4		4·5	2·9	7·6	20·0	68·1	68·6	75·5	7·12
No. 6	2·2	4·1	2·9	9·5	22·3	66·7	69·6	75·9	6·62
No. 8	3·7	7·3	11·2	41·9	72·1	74·2	75·4	76·3	9·05
No. 9	6·4	6·2	15·1	51·4	78·8†	75·6	76·3	75·9	8·39

* Total starch in polished seed determined by official method of American Agricultural Chemists.
† This figure is evidently wrong.

The proteid content of the polished seed is shown for reference.

It should be pointed out here that the determinations of liquefied starch rest finally upon a permanganate titration.

This in itself is an exceedingly delicate and accurate determination. But to deal with large quantities for the present work is difficult and thus it comes about that in the above table 1% of starch is approximately equal to 0·1c.c. potassium permanganate. We must allow therefore that the figures in the table may be inaccurate to the extent of 1% starch.

This explains partly certain small irregularities at the lowest and highest temperatures (*i.e.*, at the points where the action had scarcely commenced and where it was practically complete).

Another possible source of error may lie in the varying strength of the malt extract employed.

The malt was not specially tested, but it was prepared each day in as uniform a manner as possible from a stock sample of green malt preserved in dessicators.

That it varied very little indeed is proved by the regularity of the results obtained and by some duplicate determinations (see below Table II). The use of standardised diastase would no doubt add to the value of such results. This will be employed in the work now contemplated.

All the determinations for one temperature were made on one day with one specimen of malt.

Concerning the amylolytic power of the seeds a remark is called for.

On the unliquefied starch (at lower temperatures) its effect is clearly negligible, and therefore it can be assumed that at the higher temperatures all the starch attacked by the combined amylolytic enzymes of the malt and the rice grains must have first of all been liquefied. That is to say, the presence of such enzymes in the rice, even if they have not been entirely destroyed by the alkali, cannot materially affect the determinations of percentage liquefaction.

It will be observed that the soluble carbohydrates (sugars, etc.) have been estimated together with and as starch.

This makes no difference to the results because if they were allowed for, it would merely mean the subtraction of a constant quantity (say 1 %) from each of the figures without altering their significance in the least.

The shape of the curves in the chart on page 144 would naturally not be affected in the smallest degree by the introduction of this correction.

There is one more point to be considered.

Some of the samples apparently contain some very resistant starch (see further on). It appeared that this resistance to liquefaction and malt conversion might possibly be due not to the nature of the starch but to incomplete action caused by imperfect disintegration of the grain. This point was tested by comparing specimens disintegrated in different ways.

The results are given in the accompanying table.

TABLE II. *Effect of disintegration on fractional liquefaction at* 80°.

Sample.	Ordinary disintegration 24 hours.	Disintegration for 48 hours.	Disintegration 48 hours with crushing after 24 hours.	Average.
No. 1	70·0	68·6	69·4	69·3
No. 2	69·4	66·7	68·1	68·1
No. 3	67·7	65·7	66·7	66·7
No. 4	70·0	67·7	68·1	68·6
No. 6	70·4	69·0	69·4	69·6
No. 8	77·1	74·2	75·0	75·4
No. 9	76·3	75·6	77·1	76·3

Clearly the ordinary disintegration process cannot be improved upon and therefore it seems fairly well established that the so-called resistant starch does actually exist. (*Cf.* Wolff and Fernbach.)

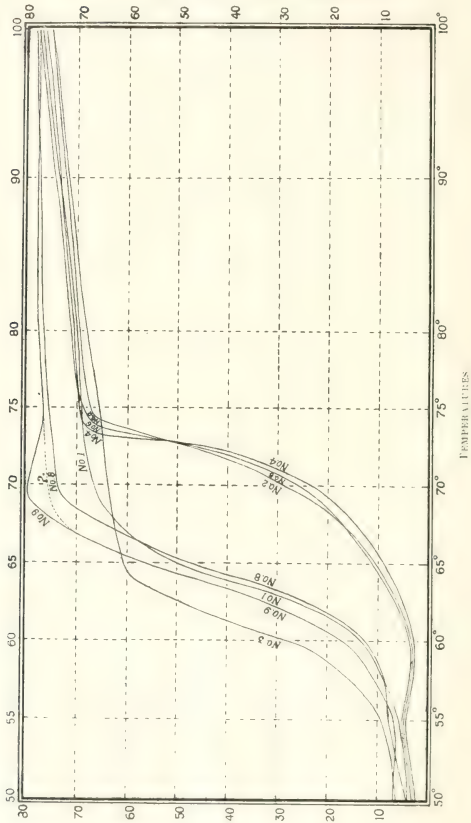
The 3 sets of determinations were made each with a separately prepared malt extract.

The table shows also therefore that the malt extracts were all very nearly equal in diastatic power.

It remains now to discuss the significance of the results obtained.

The chart on the back is a graphical representation of the series of determinations.

The three specimens 2, 4 and 6 are hard vitreous rices. The extreme similarity of their starches is remarkable.


PERCENTAGE LIQUEFACTION OF STARCH.
TEMPERATURES
No 4
No 2
No 5
No 1
No 6
No 3

Up to 65° they are liquefied only to a slight extent. At 70° when the other rice starches have become almost completely liquefied these three show a liquefaction of only 20%. Within the next 5 degrees, however, almost all their starch is liquefied. The sudden break-down indicates a large proportion of very uniform material.

It should be noted that in each case there is a residue of 7 or 8% of more resistant starch which only breaks down gradually.

Here the great advantage of fractional liquefaction is clearly seen.

Methods which only indicate the temperature of complete liquefaction would scarcely enable us to distinguish between samples 1 and 4 for example, whilst by fractional liquefaction a great difference is brought to light.

Of the other specimens Nos 8 and 9 are true opaque white glutinous rices.

The starch of these specimens commences to liquefy at a much lower temperature. It is noteworthy also that these two specimens contain practically no trace of resistant starch, the liquefaction being almost complete between 70° and 75°.

No. 3 is a remarkable specimen. Its liquefaction is already far advanced at 60°. But after 65° there is very slow progress indeed and eventually it is surpassed by all the other rices.

This variety contains a fraction of very easily liquefied starch, together with 8% of more resistant starches which are liquefied in small fractions as the temperature is increased above 70°.

No. 1 is considered by the Burmans to be a semi-glutinous variety not very suitable for cooking.

Its intermediate character is revealed by its partial liquefaction at lower temperatures (similar to glutinous rice) and by the presence of a resistant fraction (similar to the hard rices).

It is grown more for sale and export than for home consumption.

That the cooking quality of rice is distinctly correlated with its starch quality is clearly proved by these results.

Attention has been called in a previous paper* by us to the fact that rice varieties could be distinguished from one another by disintegration with alkali.

To determine how far such disintegration is correlated with the quality of the starch these rices were treated with alkali as already described.

The result is shown in the Plate.

It is evident that the disintegration does roughly correspond with the starch quality.

The three hard rices Nos. 2, 4 and 6 are not so easily attacked as the other specimens.

Again No. 3 which contains much easily liquefied starch is also most easily disintegrated.

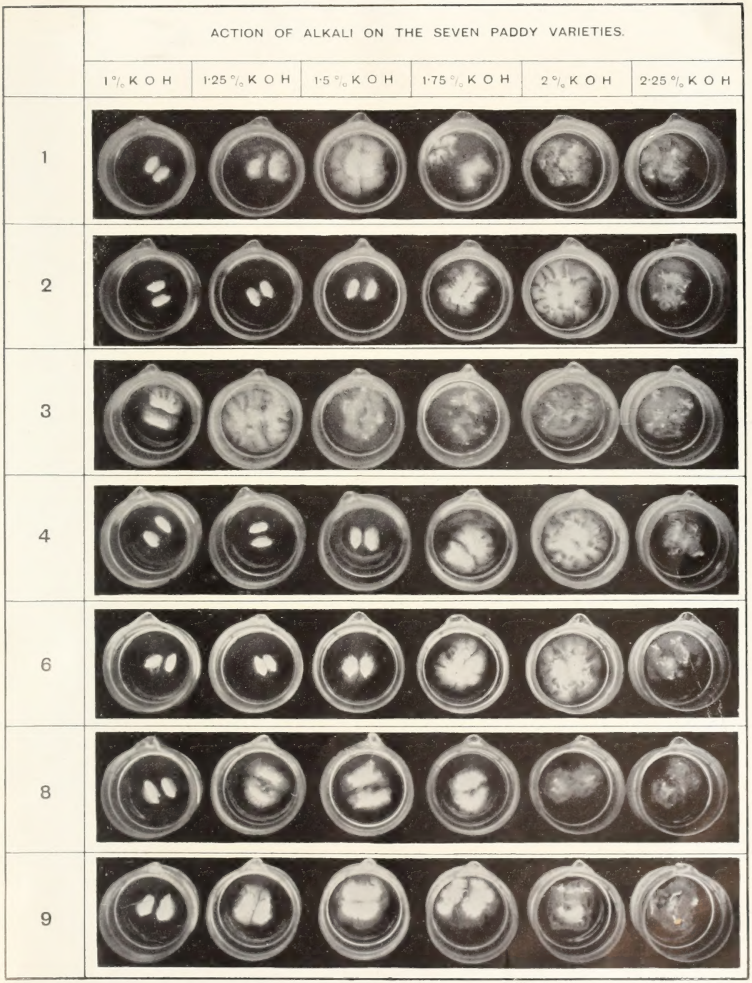
Obviously the ease of disintegration cannot depend upon starch quality alone. Indeed the earlier stages of the process (at low concentrations of alkali) are probably quite independent of the starch quality. The parallelism found to exist between disintegration and starch quality therefore seems in some cases at least to indicate a relationship between starch quality and proteid quality. To establish such a relationship, if it exists at all, will demand observations on many more samples extending over several years.

It should be noted that the parallelism referred to above is, as might have been expected, far from complete in every case. This may be seen by comparing for instance the disintegration of samples 1, 8 and 9 with the corresponding liquefaction data.

The work described in this paper is being continued to determine the effect of environment upon starch quality.

A reference to Table I shows that there is no very obvious relationship between quality of starch and quantity of proteid. Some light may possibly be shed on this point also when the effect of environment is studied.

* Watts and Parabisetti, *Pusa Agri. Res. Inst. Bull.* 38.


ACTION OF ALKALI ON THE SEVEN PADDY VARIETIES.
1 % K O H
1·25 % K O H
1·5 % K O H
1·75 % K O H
2 % K O H
2·25 % K O H
1
2
3
4
6
8
9